Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

BULLETIN
OF THE
TORREY BOTANICAL CLUB.

Vol. XIX.] New York, July 13, 1892. [No. 7.

Observations on some American Rhizobia.

By A. Schneider, University of Minnesota.

Plates CXXIX and CXXX.

The purpose of this paper is: first, to give a short review of "Pilzsymbiosis;" second to point out and discuss somewhat the existing controversies; and third, to show that there are probably more than one species of *Rhizobium.*

It is only within recent years that the symbiotic relations of fungi with plants have been discovered and studied. Half a century ago some of the fungi which are now known to be symbiotic were studied and described without suspecting their real nature. Theo. Hartig in 1840 made drawings and gave a description of the "mykorhiza" of *Pinus sylvestris*, taking it to be a purely parasitic fungus. With this in view, at the "Botanischer Verein in München," Nov. 11, 1885, R. Hartig criticises Frank for claiming to have discovered the symbiotic relations of *Rosellinia quercina* with the oak To my knowledge nothing of any moment had been done on Pilzsymbiosis prior to 1885. B. Frank no doubt is the most earnest worker and has done more than any other man to clear up some of the mysteries concerning the subject. Probably he often becomes too enthusiastic over his discoveries, claiming for instances that many trees, especially of the Cupuliferæ, cannot develop without the symbiotic fungi. R. Hartig seems to be of the opposite turn of mind. In his "Anatomie und Physiologie der Pflanzen," 1891, he sums up Pilzsymbiosis as follows: "Fungi probably belonging to the Tuberaceæ or Gastromyceteæ are found parasitic on, or symbiotic with certain trees, especially the Coniferæ and Cupuliferæ. In some cases they form a true mycorhiza (fungus root)." He does not seem to think that these fungi are of any use to the plant, but, on the contrary, more or less harmful by taking nourishment properly belonging to the plant. The hypertrophic condition of the infested root is probably caused by an irritating substance secreted by the fungus. But many forest trees of different varieties showed no traces of the root fungus.

In 1887 Hellriegel and Willforth concluded a long series of experiments which led to a new hypothesis in regard to the free-nitrogen-assimilating function of the Leguminosæ. Notes on these experiments dated back from the years 1862 and 1863. They were conducted under the direct supervision of Hellriegel. Among his assistants were H. Roemer, R. Günther, H. Moeller and G. Wimmer. Without going into details their conclusions may be summed up as follows:

1. Leguminous plants develop in sterilized soil.

2. Addition of sterilized nitrates increased their growth.

3. In harvest time there was no excess of stored nitrogenous material which could have come from any other source than the soil.

4. In non-sterilized soil the Leguminosæ developed better than in sterilized soil, especially if there was added a solution made from soil in which leguminous plants had grown (culture soil solution).

5. In harvest time there was found stored up in the plant an overplus of nitrogenous compounds which could not have been taken from the soil.

6. Leguminous plants in sterilized soil to which only a small amount of the above mentioned culture soil solution was added showed this overplus of nitrogenous compounds.

7. One and the same Leguminous variety did not give like results with different culture soil solutions.

8. Leguminosæ in non-sterilized or sterilized soil inoculated with the culture soil solution developed tubercles.

9. Leguminosæ in sterilized soil developed no tubercles.

10. In the same plant, if one side of the root was dipped in

normal culture soil solution and the other in sterilized culture soil solution the former developed tubercles, the latter not.

11. A period of dwarfed growth was noticed after inoculation with the culture fluid.

12. During this period tubercles kept on developing.

13. In soil free from nitrates active growth began only after the formation of tubercles.

Hellriegel's final summing up is as follows:

1. The Leguminosæ have another source besides the soil from which to draw free nitrogen.

2. This second source is the free nitrogen of the air.

3. The plant itself has not the power to assimilate this free nitrogen.

4. Active micro-organisms perform this function for the plant.

5. The tubercles stand in intimate relations with the free nitrogen assimilating power of the Leguminosæ.

In 1890 Frank wrote an article entitled "Pilzsymbiose der Leguminosen" in which he not only verified Hellriegel's conclusions, but also studied and described the micro-organisms connected with this nitrogen-assimilating function. His conclusions may be briefly summed up as follows:

1. Some Leguminosæ live in symbiotic relations with a very simple fungus which infects the plant as soon as it begins to develop.

2. This fungus belongs with the smallest organisms. It is one of the Schizomycetes, the *Rhizobium leguminosarum*.

3. It very likely develops and multiplies in the soil. It is present in all natural soils, at least to some extent.

4. Roots of the Leguminosæ have the property of excreting a peculiar substance which attracts the spores of the fungus. Some of these spores develop on the outside of the root, others are conducted into the interior of the root tissue by peculiar protoplasmic strands built up by the normal cells of the plant (*Infektionsfäden*).

5. The fungi combine with the protoplasm of the cells, forming a mixture called "mycoplasm." The fungus may be found in nearly all parts of the plant, even in the seed. A new plant may be infected from the spores in the embryo.

6. At the point of infection a tubercle develops. This consists of parenchymatous tissue rich in protoplasm in which the spores develop and multiply very rapidly.

7. The mycoplasm becomes differentiated into nitrogenous (albuminous) "Formelemente"—the "Bakteroiden." These resemble bacteria.

8. At the close of vegetation these stored bacteria-like collections of albuminous substances are again absorbed and utilized by the plant.

9. Some tubercle-producing fungi of the Leguminosæ are purely parasitic; that of *Phaseolus vulgaris* probably belongs to this group.

10. In most Leguminosæ the *Rhizobia* have the power of appropriating for the use of the plant free nitrogen of the air.

11. There is probably only one species of the *Rhizobium* which is present in all normal soils.

12. Leguminosæ in rich soil develop few, if any tubercles. Plants in poor soil stand in closer symbiotic relations with the *Rhizobia.*

Frank's conclusions in regard to Pilzsymbiosis as they appear in Heft 7, 1891 of the "Berichte der Deutschen Botanischen Gesellschaft" may be briefly summarised as follows:

1. Endotrophic mykorhiza is found in roots of Orchids, Ericaceæ and other plants.

2. In the Orchids the mykorhiza consists of a collection of yellowish interwoven septate hyphæ, generally found in epidermal cells of the root or rhizome.

3. The fungus during its whole life-course lives within the root cells of the host.

4. Nuclei of infected cells become much enlarged, showing an increased activity of cell protoplasm.

5. The living protoplasm of the root cells absorbs the protoplasm of the fungus. This is observed in roots that approach the period of decay.

6. The fungus is at first nourished by the cell protoplasm, which changes it so as to render it unable to grow outside of the root cell.

In regard to the mykorhiza of Ericaceæ, Frank comes to similar conclusions. His pupil Schlicht has found fungi in roots of the following families. Ranunculaceæ, Leguminosæ, Rosaceæ, Œnotheraceæ, Umbeliferæ, Geraniaceæ, Oxalidæ, Hypericaceæ, Violaceæ, Primulaceæ, Labiatæ, Boragineæ, Dipsaceæ, Valerianaceæ, Plantagineæ, Rubiaceæ, Smilaceæ, Compositæ, Gramineæ. The true nature of the *Rhizobia* in these numerous families is not known in most cases.

In regard to symbiosis of the Leguminosæ in the same article Frank comes to the following conclusions:

1. At least some of the Leguminosæ live in symbiotic relations with a fungus with which the plant becomes infected as soon as it begins to develop in normal soil.
2. This fungus has specific properties belonging to the Schizomycetes; it is the *Rhizobium leguminosarum.*
3. The fungus probably develops and multiplies to some extent in the soil.
4. The roots of the Leguminosæ have the power of attracting the fungus. This is probably due to some peculiar secretion.
5. The manner of infection is the same as described in 1890.
6. The "Bakteroiden" of 1890 are now considered true fungi. They are, however, much modified through the assimilation of cell protoplasm.
7. These modified fungi will not multiply outside of the vegetable cell.
8. A few normal fungi are always left over, which pass into the soil ready to infect other developing plants.
9. There is only one species of *Rhizobium.*

In comparing Frank's résumé of 1890 with that of 1891 the following differences are noted: the Bakteroiden are not albuminous differentiations of the mycoplasm, but they are considered to be true, much-altered *Rhizobia* and these modified *Rhizobia* cannot grow and multiply outside of the vegetable cell. He admits that in his former decision in favor of Bakteroiden he had been influenced by Brunchorst. Woronin in 1866 was the first who maintained that the Bakteroiden of Brunchorst were true bacteria. Indeed, Frank thought for a time that all the controversy about Bakteroiden being living organisms was due to Woronin. In the last year or two the strongest arguments are with Frank's latest decision (1891). M. Ward took the Bakteroiden to be the spores of a true hyphal fungus. Ericksson also took them for a spore or bacterioid buddings from true hyphæ.

The "Infectionsfäden" seem to be another cause of much controversy. Frank formerly likened them to the *Schinzia* (*Plasmodiophora*) *Alni* of Woronin and called them *Schinzia leguminosarum*. As will be seen from his conclusions of 1890 and 1891 he definitely states that they are not a fungus, but a production of the normal plant cells used for the purpose of infection. M. Ward took the Infektionsfäden for the real fungus of which the Bakteroiden were the spores. Prazmowski at first took them for plasmodia filled with small rod-like bodies. As soon as the plasmodium enters the root cells it surrounds the entire cell contents so that plasmodium and cell contents can not be distinguished. Later he changes his opinion and takes the Infektionsfäden for hyphæ-like tubes filled with bacteria. That is, the hyphæ-like tubes, if not identical with Frank's Infektionsfäden, at least have a similar function. Schroeter seems to be satisfied that the Infektionsfäden are true plasmodia made up of swarm spores of his *Phytomyxa leguminosarum.*

At present I shall not enter into any further discussions of existing controversies, but shall proceed with my own observations and resultant conclusions. In my observations I studied the *Rhizobia* as I found them in the various Leguminosæ. Frank and Beyerinck, so far as I can learn, made their principal observations on culture *Rhizobia.* These cultures are apt to lead to erroneous results for three reasons: First, because but little is at present known of their behavior outside of their host; second, because the same tubercle may contain a variety of *Rhizobia* and other bacteria, especially tubercles that have begun to break down—these would be liable to influence the culture formations; third, because of the well known fact that culture fluids greatly modify the appearance of bacteria. Frank seems to give no plausible reason why there should be but one species of *Rhizobium.* Frank and Beyerinck both admit that this one species occurs in a variety of forms, Y-shaped, oblong, curved, etc. In the examination of normal culture soil solution I found not only a variety of *Rhizobia,* but also a host of other bacteria, bacilli and micrococci. That all *Rhizobia* may have a similar function, that of assimilating free nitrogen, does not indicate that there must be but one species. Even since Frank maintains that the Bakteroiden are true, though modified, *Rhizobia* he seems to believe that there is but one species. In 1890 he declared that the micrococci-like *Rhizobia* were contained within the Bakteroiden which, according to his recent conclusions in regard to Bakteroiden, would be absurd or at least improbable.

Frank also seems to take it for granted that the plant or rather root has some attractive influence on the *Rhizobia.* He gives no cause for this assumption, nor do experiments bear him out. Capillarity, wind and water are the principal agents for the distribution of micro-organisms in soil. Air currents carry them from place to place on the surface of the soil. Rain, for instance, carries them deeper into the soil and brings them in contact with roots and rootlets; these with their root hairs and probably a sticky secretion, arrest them and infection takes place at suitable points. Capillarity will either carry them down or up, that depending upon the condition of the soil as to moisture. Hellriegel and Beyerinck have shown that when a leguminous plant was planted in sterilized soil and one side sprinkled with normal culture soil solution and the other with sterilized culture solution, the former developed tubercles while the latter did not. Now if the roots had any attractive influence they would certainly have drawn some of the *Rhizobia* from the infected to the non-infected side.

The purpose of my observations was primarily to settle the following questions:

(1). Do all the tubercles of the Leguminosæ contain one and the same micro-organism?

(2). Do all the micro-organisms undergo a modification of form in all infected Leguminosæ?

(3). Is the micro-organism a *Rhizobium* or a *Phytomyxa ?*

In May, 1891, I examined the tubercles of *Trifolium pratense* and *T. repens* and noted the appearance of the contained *Rhizobia.* They were as shown in fig. 1, plate CXXX. Later, in June and July I examined tubercles of *Amphicarpaea comosa, Cassia Chamæcrista, Trifolium repens, T. pratense, Melilotus alba, Pha-*

seolus vulgaris, *P. pauciflorus*, *Lathyrus odoratus*, *Pisum sativum* and *Robinia Pseudacacia*. According to the statements of Frank I expected to find in each species examined the same kind of *Rhizobium*, though perhaps somewhat modified. This, however, was not the case. In *Melilotus alba*, *Trifolium repens*, *T. pratense* and *Lathyrus odoratus*, I found the cells of the infected area of tubercles entirely filled with the peculiar Y-shaped organisms, (the "Bakteroiden" of Brunchorst, bacteria of Woronin, spores or gemmules of Ward, etc.) (fig, 1, plate CXXX.) The same tubercles examined in June and July showed the same organisms, though much changed in appearance. They were larger, and the majority took on the Indian-club form. They had become fattened on the cell protoplasm (fig. 2, plate CXXX). The same tubercles examined in September and October showed the organisms still more modified. Some of them had become almost spherical, others oval-dumb-bell shaped, etc. In some the plant had begun to reabsorb the protoplasmic contents, as shown in *a*, fig. 3, plate CXXX. The same tubercles, examined late in October and beginning of November, showed that most of them had begun to break down, and the contained *Rhizobia* had their entire protoplasmic contents reabsorbed by the plant, leaving nothing but the empty, partially absorbed or decayed walls (fig. 3, plate CXXX). In these tubercles were also found a variety of bacteria spores and *Rhizobia* (?) that were not present while the tubercle was intact. Very likely these were foreign to the plant, and had been brought to and deposited in the partially destroyed tubercle by water currents and capillarity. Probably these foreign spores, bacteria and *Rhizobia* (?) were the organisms which Frank took to be the unmodified remnants and spores of the true *Rhizobium leguminosarum* left behind after the protoplasmic contents had been reabsorbed by the plant. Several culture attempts on gelatine in July proved to be failures. This confirms Frank's statements that the modified *Rhizobia* will not reproduce and develop outside of the host. Neither by the aid of various stains or the use of K H O solution was I enabled to detect the presence of spores. I am inclined to the belief that they multiply by direct division only.

In the examination of the tubercles of *Phaseolus pauciflorus* I found the cells of the infected area entirely filled with curved *Rhizobia*, resembling the comma bacillus of Koch. Staining with eosin or fuchsine brought into view a spore at either end. Their form was so constant and even that I could not bring myself to imagine that they were identical with the *Rhizobia* of *Melilotus alba*, for example. Furthermore they retained their constant form throughout the entire year. The *Rhizobia* of *Phaseolus vulgaris* were still different—they were straight, in form constant, in size quite variable. There were spherical spores, one at either end, rarely only one. The spores of the curved *Rhizobia* were hemispherical. The *Rhizobia* of *Pisum sativum* corresponded exactly with those of *Phaseolus vulgaris*, only that they were smaller. *Dalea alopecuroides*, *Robinia pseudacacia* and *Cassia Chamæcrista* contained a *Rhizobium* differing from any of the ones described above. It presented a knotted, irregular outline; generally three spores, sometimes one, rarely two. The *Rhizobia* of *Amphicarpæa comosa* differed from the one just described in that the spores were placed some distance from the ends. They were also somewhat smaller, as will be seen from the table of measurements. From these observations I felt satisfied that there was more than one species of *Rhizobium*, and that they did not all take part in that change of form so well marked in the first described species. The question might be raised whether the protoplasmic contents of the *Rhizobia* are in all cases reabsorbed by the plant or whether they, at least in some cases, simply die and disintegrate for want of food supply. It is known that in all cases the tubercles are destroyed towards the close of vegetation, thus liberating the remaining *Rhizobia*.

As already stated, Schroeter took the Infektionsfäden to be true plasmodia of his *Phytomyxa leguminosarum*,=Frank's *Rhizobium*. Now the Infektionsfäden are very prominent in *Trifolium pratense* but its *Rhizobia* contain no spore whatever so far as I have been able to make out, so the Infektionsfäden cannot be the plasmodium of the *Rhizobium* of white clover. Furthermore the Infektionsfäden are absent in many species infected with the *Rhizobia* or *Phytomyxa*. So instead of placing the *Rhizobia* with *Myxomycetes*, according to Schroeter, it is better to place them with the Schizomycetes, according to Frank; because their composition, development, methods of reproduction and general behavior correspond to those of the Schizomycetes.

Frank claims to have seen the spores of *Rhizobium* within the Infektionsfäden. I have been unable to find anything in them except a granular, spongy (*schaunmiges*) appearance when treated with K H O solution. These Infektionsfäden are not present in all tubercles, for instance, those of *Phaseolus vulgaris*, *Pisum sativum*, *Robinia Pseudacacia.* Nor are the "infecting strands" always absent where Frank thinks they are not needed. I have found them in young *Phaseolus vulgaris;* have traced them in tissues opposite the tubercle, or some distance from the tubercle, having no connection with it. Nor do these "strands" generally originate in a root hair, but more often from the epidermal layer of root cells, where they begin as a small spherical protoplasmic lump, as shown at *a*, fig. 1, plate CXXIX. These infecting strands seem to have no outer wall; they are nonseptate, branching, interlacing, passing directly through the protoplasm and wall. Their protoplasm is of a different consistency from that of either the cell or *Rhizobia.* It is more resistant toward chemicals and staining fluids. It remains after the protoplasm of cell and *Rhizobia* have been used up. It is more than likely that these Infektionsfäden have nothing to do with the *Rhizobia;* because, they are not present in all cases, and when present, are not always in or near tubercles. I could detect no spores in them. They are present in tubercles of *Trifolium repens*, whose *Rhizobia* have no spores. I found them in young *Phaseolus vulgaris*, where Frank says they are not needed. Since infection in some cases takes place without the "strands," may it not do so in all cases?

The form of the tubercle seem to have nothing to do with the kind of infecting *Rhizobia.* In *Dalea alopecuroides* and *Lathyrus odoratus*, for instance, are found similar tubercles as to form and size, but they contain distinct *Rhizobia.* I did not notice much regularity as to the position and distribution of tubercles. They are usually more numerous near the surface of the ground. They may be found on the main root or on its branches. In *Trifolium repens* and *T. pratense* they seem to form more often where rootlets branch. The root hairs on tubercles are usually few and dwarfed, as shown in fig. 1, plate CXXIX.

The histology of the tubercle may be briefly considered as follows:—The tubercle consists of a collection of abnormally large parenchyma cells whose protoplasm and nuclei are much modified: The nuclei are much enlarged, chromatine prominent, *Rhizobia* imbedded in the cell protoplasm, all division very active, thus producing new and infected cells. Beginning on the outside in examining a section of tubercle, the following layers may be described. Epidermal layer, no root cap; non-infected area one, two or more layers thick; immediately below that is the starch-bearing zone entirely surrounding the infected area, and continuous with the starch-bearing layer of the vascular bundle. The remainder of the tubercle is the infected area filled with *Rhizobia* and some starch granules. No starch is present in cells entirely filled with the *Rhizobia.* The plant no doubt requires the protoplasm of the *Rhizobia* from which to build starch. Between the infected and non-infected area, and including the starch-bearing zone, the cells are partially filled with *Rhizobia*, (see fig. 1, plate CXXIX).

Tubercles had been noticed by some of the older botanists. Malphighi took them for animal galls; Treviranus (Bot. Zeit., 1853) for adventitious buds; DeCandolle for pathological growths; Clos for lenticels. Their real nature was not suspected.

SYSTEM OF "RHIZOBIA."

SCHIZOMYCETES.

MYCODOMATIÆ.

Rhizobia developing and living in root tubercles of variable size and form. Tubercles always on main root or its branches, and decaying at the close of vegetation.

Rhizobium mutabile, n. sp.

Syn.: *Bakteria*, Woronin.
Bakteroiden, Brunchorst.
Spores or gemmules, Ward.
Bacillus radicicola, Beyerinck in part.

This species was first discovered and probably the most common. It may be that this species, above all others, has the power to assimilate free nitrogen. Experiments will be necessary to determine that point. As is seen from the synonyms, some authorities took this species for an organism and others did not. Woronin was the first to place it among living organism, the bacteria. Beyerinck believed that they were modified bacilli. Ward maintained that they were the budded-off gemmules or spores of a true hyphal fungus, as already explained. Brunchorst, and also Frank, said they were bacterioid collections of albuminios substances formed from the cell protoplasm. Recently Frank claims that they are true *Rhizobia*, because plasmolysis causes them to contract like living protoplasm.

I have found this species symbiotic with *Trifolium pratense* and *T. repens*, *Melilotus alba* and *Lathyrus odoratus*. It is at once recognized by its size and the varying form it takes on during the vegetation period. It was impossible for me to detect the presence of spores during any time of its existence. It very likely multiplies by division only. The tubercles produced are oblong or irregular, varying in size from that of a pin head to that of a small pea; sometimes they are collected in large grape-like bunches, as in *Melilotus alba*. The modified organisms do not develop upon gelatine or agar-agar cultures. They are surrounded by a gelatinous zone.

Rhizobium curvum, n. s.

Syn.: *Rhizobium leguminosarum*, Frank.
Phytomyxa leguminosarum, Schroeter.
Cladochytrium leguminosarum, Vuillemin.
Bacillus radicicola, Beyerinck.
Pasteuriaciæ, Laurent.
} in part.

Symbiotic with *Phaseolus pauciflorus*. Cells curved, resembling the comma bacillus of Koch. One hemispherical spore at each end, rarely more. Size quite uniform, and form constant during entire vegetative period. Gelatinous zone present. Tubercles spherical, varying in size from that of a pea to that of a small hazel nut.

Rhizobium Frankii, n. s., var. *majus*, n. v.

Syn.: Same as preceding.

Symbiotic with *Phaseolus vulgaris*. Cells straight, generally two or three times as long as broad. Size variable. Two spherical spores, one at either end, sometimes one, rarely none. Spores larger than in the preceding. External gelatinous coat. Tubercles small, spherical, comparatively few in number.

Rhizobium Frankii, var. *minus*, n. s.

Syn.: Same as preceding.

Symbiotic with *Pisum sativum.* It is smaller; otherwise like the preceding.

Rhizobium nodosum, n. s.

Syn.: Same as preceding.

Symbiotic with *Dalea alopecuroides*, *Robinia Pseudacacia* and *Cassia Chamæcrista.* Cells of an irregular, knotted appearance. Protoplasmic contents often collected in lumps. Generally one or three, rarely two, spores situated at the ends, with one somewhere near the middle. Sometimes an encapsuled chain of spores. Gelatinous zone present. Tubercles round, oblong or irregular, varying in size from that of a morphine pill to a large pea.

Rhizobium dubium, n. s.

Syn.: Same as preceding.

Symbiotic with *Amphicarpæa comosa.* General description corresponds with the preceding, only that it is somewhat smaller and the spores are not situated in contact with the end walls as in all the preceding; but small distances from them. Tubercles spherical, about half as large as a pea. Considerable starch in tubercles.

For better understanding of the above described species consult table of measurements and plate CXXX.

TABLE OF MEASUREMENTS.

	LENGTH.	WIDTH.
	μ	μ
Rhizobium mutabile in May,	3-4	.8
“ “ “ July,	6.4	1
“ “ “ September,	5-8	2-3.5
“ *curvum*,	1.9	.6
“ *Frankii*, var. *majus*,	.8-3	.6
“ “ “ *minus*,	.6-1.4	.5
“ *nodosum*	1.6-3.5	.8
“ *dubium*	1.6-2.4	.6

Settled Facts.

1. That certain fungi are symbiotic in and on roots of certain plants.
2. Some of these fungi are purely parasitic, while others have the power of assimilating for the use of the plant free nitrogen of the air.
3. Plants can exist without these fungi, but as a rule thrive better with them.
4. At the close of vegetation, and also on other special occasions, the plant reabsorbs the protoplasm of the fungi.
5. The Infektionsfäden are not hyphæ of which the Bakteroiden are the spores or gemmules.

Probable Facts.

1. That there are at least several species of *Rhizobium.*
2. That the Infektionsfäden have nothing to do with the *Rhizobia* under consideration.
3. That the *Rhizobia* belong to the Schizomycetes.
4. That *Rhizobium mutabile* has no spores.
5. That the Infektionsfäden are true hyphal fungi allied to *Schinzia Alni.*

Improbabilities.

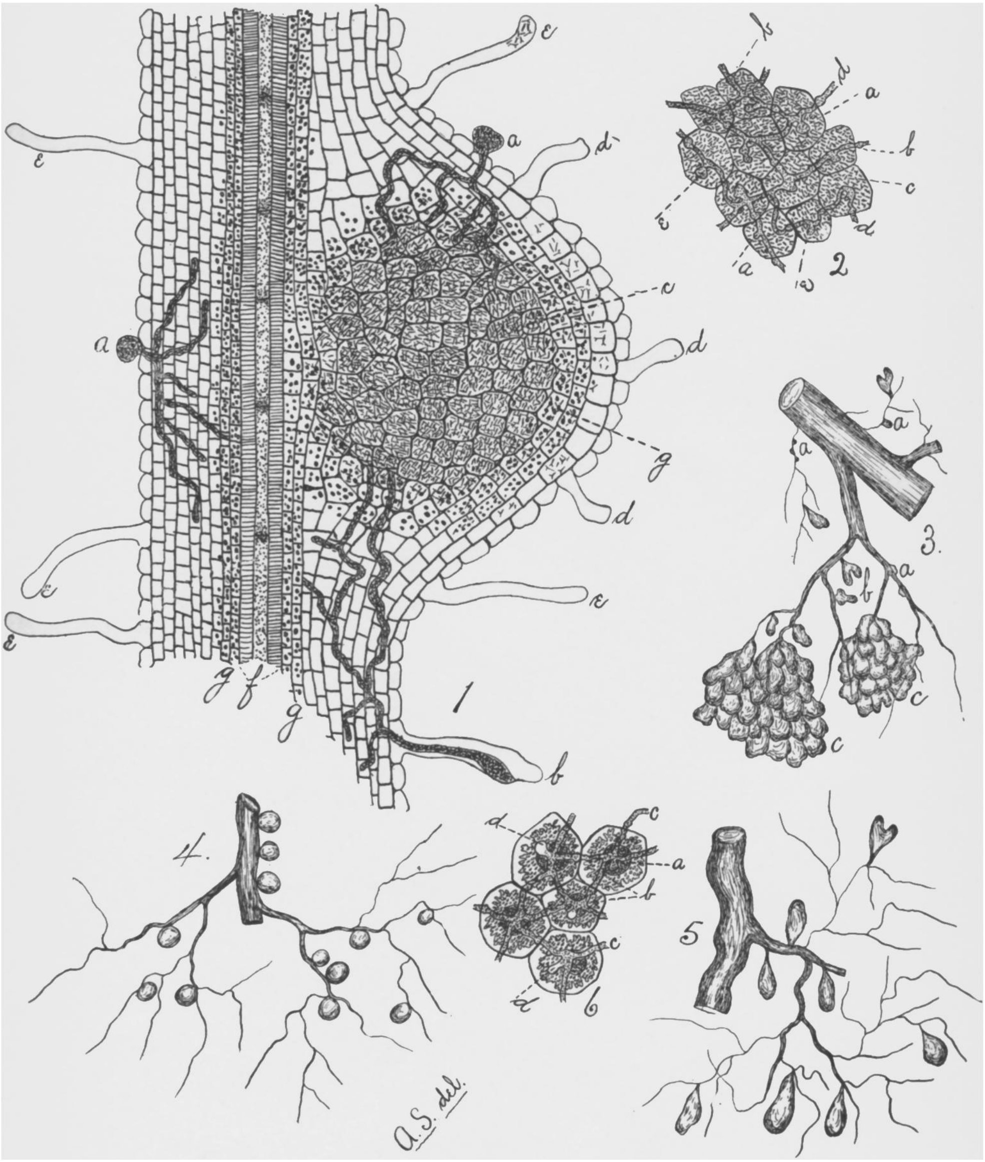
1. That the Infektionsfäden are plasmodia.
2. That the *Bakteroiden* are not living organisms.
3. That the plant exerts any attractive influence upon the *Rhizobia.*
4. That some plants cannot exist without the symbiotic fungus.

The researches of which this paper is a partial record, were undertaken in 1890 and 1891, in the Laboratories of Botany, at the University of Minnesota, in Minneapolis.

BIBLIOGRAPHY.

Benecke, F. (Botanisches Centralblatt, 1887, No. 2).
Beyerinck, M. W. (Botanische Zeitung, 1890, No. 52).
" " (Botanisches Centralblatt, 1891, No. 8).

BULLETIN OF THE TORREY BOTANICAL CLUB. Plate CXXIX.



Some American Rhizobia. A. Schneider.

Breal (Botanischer Jahresbericht, 1891, Heft 2).
Frank, B. (“ “ “ “ 2).
“ (“ “ “ “ 1).
“ (Ber. d. Deutsch. Bot. Ges., 1890, Heft 8).
“ (“ “ “ “ “ 1890, “ 9).
“ (“ “ “ “ “ 1891, “ 7).
“ and Otto, R. (Ibidem, 1890, H. 9).
“ (Pilzsybiose der Leguminosen, 1890).
Hartig, R. (Botanisches Centralblatt, 1886, Seite 350).
“ (Anatomie and Physiologie dei Pflanzen, 1891).
Hellriegel and Willforth (Stickstoffnährung der Gramineen und Leguminosen, 1888).
Ilosvay (Botanischer Jahresbericht, 1891, H. 2).
Koch, A. (Botanische Zeitung, 1890, No. 38).
Ludwig, F. (Ber. d. Deutsch. Bot. Ges., 1890, H. 12)
Laurent, E. (Botanisches Centralblatt, 1891, No. 8).
Müller (“ “ 1886, Seite 22).
Moller (Ber. d. Deutsch. Bot. Ges., 1890, Seite 215).
Pfeiffer, A. (Centralbl. f. Bak. and Parasi'k'de, 1887, Seite 11).
Prilleux (Bot. Centralblatt, 1891, No. 8).
Prazmowsky (Bot. Centralblatt, Band 36, 1888).
Sorauer (Pflanzenkrankheiten, 2 te Auflage, 1886).
Soyka (Centralb. f. Bak. and Parasi'k'de, 1887, Seite 12).
Schroeter (Die Natürlichen Pflanzenfamilien, 1890, H. 1)
Schlicht (Botanischer Jahresbericht, 1891, H. 2).
Schloesing (Botanisches Centralblatt, 1891, No. 8).
Tschirsch, A. (Centralb. f. Bak. and Parasi'k'de, 1887, Seite 634).
Treviranus (Botanische Zeitung, 1853, S. 393).
Ward, M. (Annals of Botany, Feb., 1888).
Zopf, W. (Die Pilze, 1890).

EXPLANATION OF PLATES.

PLATE CXXIX.

Fig. 1. Longitudinal section of *Trifolium repens* rootlet with a developing tubercle; stained with water-eosin. *a*, *Infektionsfäden* of Frank, *hyphæ* of Ward and Vuillemin, *plasmodia* of Prazmowski (?) and Schroeter. One of them is opposite the tubercle, having no connection with it. One originates in a root-hair, *h*,

They are probably true hyphal fungi closely related to the *Schinzia Alni* of Woronin. *c*. Infected area. Only cell walls and the contained *Rhizobia* are shown. *d*, Dwarfed root-hairs on tubercle. *e*, Normal root-hairs, one infected with *Rhizobia*. *f*. Vascular bundle. *g*, Starch-bearing area surrounding entire infected area.

Fig. 2. Portion of infected area somewhat more magnified showing distribution of *Infektionsfäd'n*, Nuclei and cell protoplasm are not shown. *a*. Cell filled with *Rhizobia*. *d*, Infecting strands. *b*, Infektionsfäden are somewhat expanded near cell wall. *e*, *Haustoria* of Kny. Expanded knobbed extension from the strands.

Fig. 3. Rootlet of *Melilotus alba*. *a*, Developing tubercles. *b*, Single tubercles. *c*, Grape-like bunches of tubercles, slightly magnified.

Fig. 4. Rootlet of *Amphicarpæa comosa* with spherical tubercles.

Fig. 5. Rootlet of *Dalea alopecuroides*. Tubercles resembling those of *Melilotus alba* and *Trifolium pratense* and *T. repens* though somewhat larger.

Fig. 6. Portion of infected arc of *Trifolium repens* showing nuclei treated with KHO solution, and cell protoplasm (mycoplasm). *a*, Nucleus enlarged. *b*, Cell protoplasm (mycoplasm). partially used by the plant containing modified *Rhizobium mutabile*. *c*, *Infektionsfäden*. They seem to be entirely independent of cell nuclei and mycoplasm. *d*, *Haustoria* of Kny. They do not appear granular while the strands do.

PLATE CXXX.

Fig. 1. *Rhizobium mutabile* as it appears in May, or the time of infection.

Fig. 2. The same in June and July.

Fig. 3. The same in September and October. *a*, A *Rhizobium* from which the plant has begun to reabsorb the contents.

Fig. 4. The same in October and November. a, Partially emptied *Rhizobia*. *b*, Entirely empty, nothing remaining but the partially destroyed walls. *c*, Normal forms which either had not undergone any change in the cell or had just gained access to the tubercle through its partially decayed distal end.

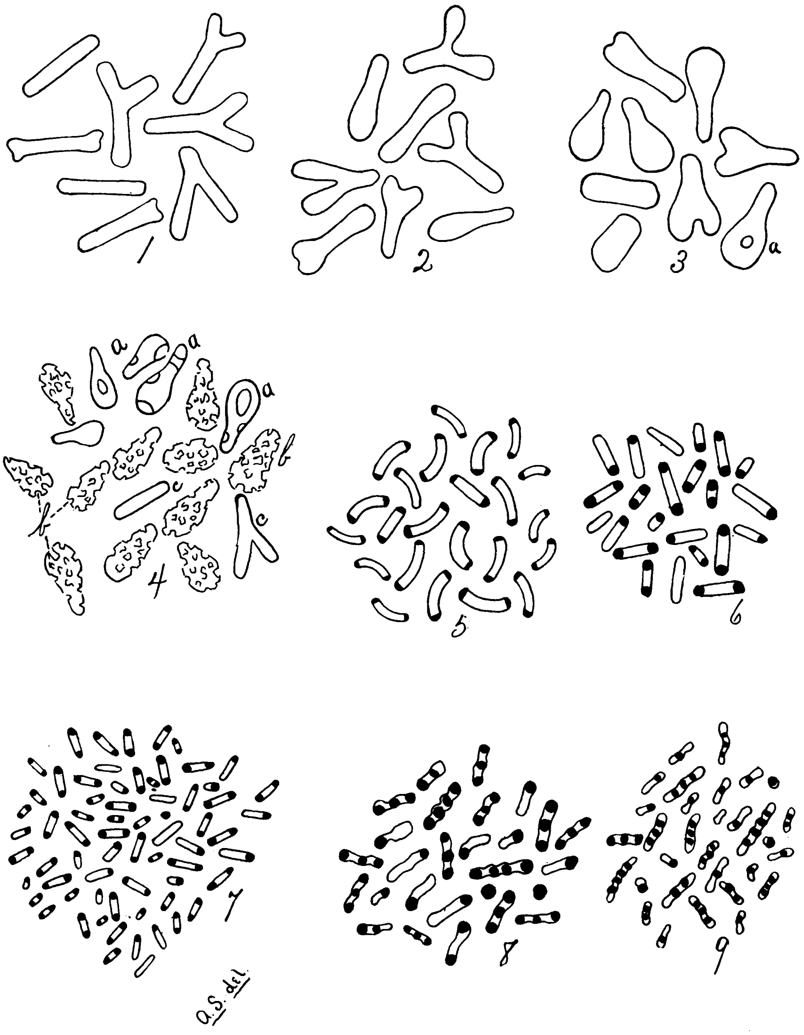
Fig. 5. *Rhizobium curvum*.

Fig 6. *Rhizobium Frankii*, var. *majus*.

Fig. 7. *Rhizobium Frankii*, var. *minus*.

Fig. 8. *Rhizobium nodosum*.

Fig. 9. *Rhizobium dubium*.



Some American Rhizobia. A. Schneider.